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Evolution

Evolutionary diversity of eukaryotic small-subunit rRNA genes

(*Euglena gracilis*/*Trypanosoma brucei*/18S rRNA sequence/evolution)

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ABSTRACT **The small-subunit rRNA gene sequences of the flagellated protists *Euglena gracilis* and *Trypanosoma brucei* were determined and compared to those of other eukaryotes. A phylogenetic tree was constructed in which the earliest branching among the eukaryotes is represented by *E. gracilis*. The *E. gracilis* divergence far antedates a period of massive evolutionary radiation that gave rise to the plants, animals, fungi, and certain groups of protists such as ciliates and the acanthamoebae. The genetic diversity in this collection of eukaryotes is seen to exceed that displayed within either the eubacterial or the archaebacterial lines of descent.**

The eukaryotes can be represented as four major divisions: the Protista, the Fungi, the Plantae, and the Animalia (1). The order of succession for these major eukaryotic lineages is an unsettled question, but it is generally accepted that the first eukaryotes were most similar to certain members of the present-day Protista. Efforts to reconstruct the evolutionary history of protists, and hence the phylogenetic origins of the major eukaryotic divisions, have been frustrated by the enormous physiological, cytological, and biochemical diversity exhibited by these "simple" microorganisms. Traditional phylogenetic analyses—i.e., examination of the fossil record and comparative studies of phenotypes—are suitable for defining relationships between multicellular organisms, but they are of limited value in determining relationships among the protists. These soft-body organisms are not well represented in the fossil record, and there is little agreement regarding which phenotypic characters are most useful for inferring protist relationships. Furthermore, comparative studies of phenotypes do not provide quantitative measures of genetic similarity that can be used to deduce the order of branching for the major eukaryotic lines of descent.

As an alternative, comparisons of the nucleotide or amino acid sequences of functionally equivalent macromolecules can be used to infer quantitative phylogenetic relationships between diverse organisms (2). Amino acid sequence homologies among cytochrome *c* molecules (3, 4), ferredoxins (5–7), and superoxide dismutases (8–10) have been useful for refining classical eukaryotic phylogenies, but they are of limited value in resolving the deepest phylogenetic branching patterns. The use of cytochrome *c* and other genes that are defined by plastid and mitochondrial genomes as "molecular chronometers" for measuring eukaryotic relationships is further complicated by their non-nuclear origin. It is now apparent that plastids and mitochondria arose as bacterial endosymbionts within some ancestral eukaryotes (11–13). Therefore, genes that are contained within the plastid or mitochondrial genomes or genes that were present in the original bacterial endosymbionts and then transferred to the nucleus—e.g., cytochrome *c* genes—should be regarded as markers of prokaryotic rather than eukaryotic evolution.

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By comparison, the rRNAs are better suited for defining evolutionary relationships. They are universally distributed and are functionally equivalent in all known organisms. Numerous phylogenies based on comparisons of the 5S and 5.8S rRNA species (14–16) have been proposed, but because of their small size these molecules do not provide statistically accurate measurements for very close or very distant relationships (12). In contrast to the 5S and 5.8S species, the small-subunit rRNAs (16–18S rRNAs) are sufficiently large to permit the statistically accurate measurement of a broad range of phylogenetic distances. These RNAs contain highly and partially conserved sequences, which can be used to measure phylogenetic relationships that span kingdoms; other regions of the molecule that display greater rates of genetic drift can be used to measure closer evolutionary distances (12).

Small-subunit rRNA sequences have been reported from two protist groups: the Sarcodina as represented by *Dictyostelium discoideum* (12) and the Ciliophora as represented by *Tetrahymena thermophila* (17) and *Stylonychia pustulata* (18). In this manuscript, we have expanded our phylogenetic analysis by determining the small-subunit rRNA gene sequences from the flagellated protists *Euglena gracilis* and *Trypanosoma brucei*. Using these sequences, we have inferred a phylogeny that provides a perspective on the evolution of the major divisions in the eukaryotic line of descent.

EXPERIMENTAL PROCEDURES

Subcloning of rRNA Genes and DNA Analysis. The recombinant plasmid pGH174, which contains the small-subunit rRNA gene for *T. brucei* (inserted into the *Bam*HI site of the plasmid pAT 153), was provided by J. S. Cordingley (19). The plasmid was grown in *Escherichia coli* strain HB-101 and amplified in the presence of chloramphenicol (30 μg/ml). The $NaDodSO_4$ alkali lysis procedure described by Maniatis *et al.* (20) was used to isolate the recombinant plasmid. A 2-kilobase-pair *Hin*dIII/*Hin*dIII subfragment together with a 1.85-kbp *Hin*dIII/*Eco*RI subfragment contain the *T. brucei* small-subunit rRNA coding region. These restriction fragments were isolated as described (18) and subcloned in the "multiple cloning" site of the M13 (mp9) single-stranded phage (21).

The *E. gracilis* small-subunit rRNA gene (inserted into the *Eco*RI site of the phage λ Charon 9) was provided by J. Rawson (22). The recombinant phage were propagated in liquid cultures of NZY medium (per liter: 1.0 g of $MgCl_2$/5 g of NaCl/10 g of NZ-amine/5 g of yeast extract) in *E. coli* strain K-802 (22) and isolated as described by Williams and Blattner (23). The *E. gracilis* small-subunit rRNA coding region resides on a 6.1-kilobase-pair *Hin*dIII/*Hin*dIII subfragment that was subcloned into the M13 (mp18) multiple cloning site. M13 templates containing the coding and noncoding strands of the small-subunit rRNA genes were sequenced by using a modification of the dideoxynucleotide chain-termination protocols (24) as described (18). Synthetic oligonucleotides (25)

that are complementary to phylogenetically conserved small-subunit rRNA sequences were used to prime the sequencing reactions.

Homology Calculations and Tree Construction. The alignment methods that were used to juxtapose evolutionarily homologous regions in the small-subunit rRNAs and the methods for inferring phylogenetic trees from structural similarity calculations are detailed elsewhere (18). Similarity values were calculated from those regions of the small-subunit rRNAs that display obvious structural similarity in all of the compared molecules. Approximately 1130 positions were considered in the analysis.

RESULTS

Fig. 1 displays the sequences for the small-subunit rRNA coding region from *E. gracilis* and *T. brucei* aligned with that of *D. discoideum*. The alignments were influenced by 18S rRNA sequences not shown in the figure from rat (26), rabbit (27), *Xenopus laevis* (28), *Artemia salina* (29), *Zea mays* (30), rice (31), *Acanthamoeba castellanii* (unpublished data), *Saccharomyces cerevisiae* (32), *Paramecium tetraurelia* (unpublished data), *Tetrahymena thermophila* (17), *S. pustulata* (18), and *Oxytricha nova* (18). The putative terminal sequences of the *T. brucei* and *E. gracilis* small-subunit rRNAs

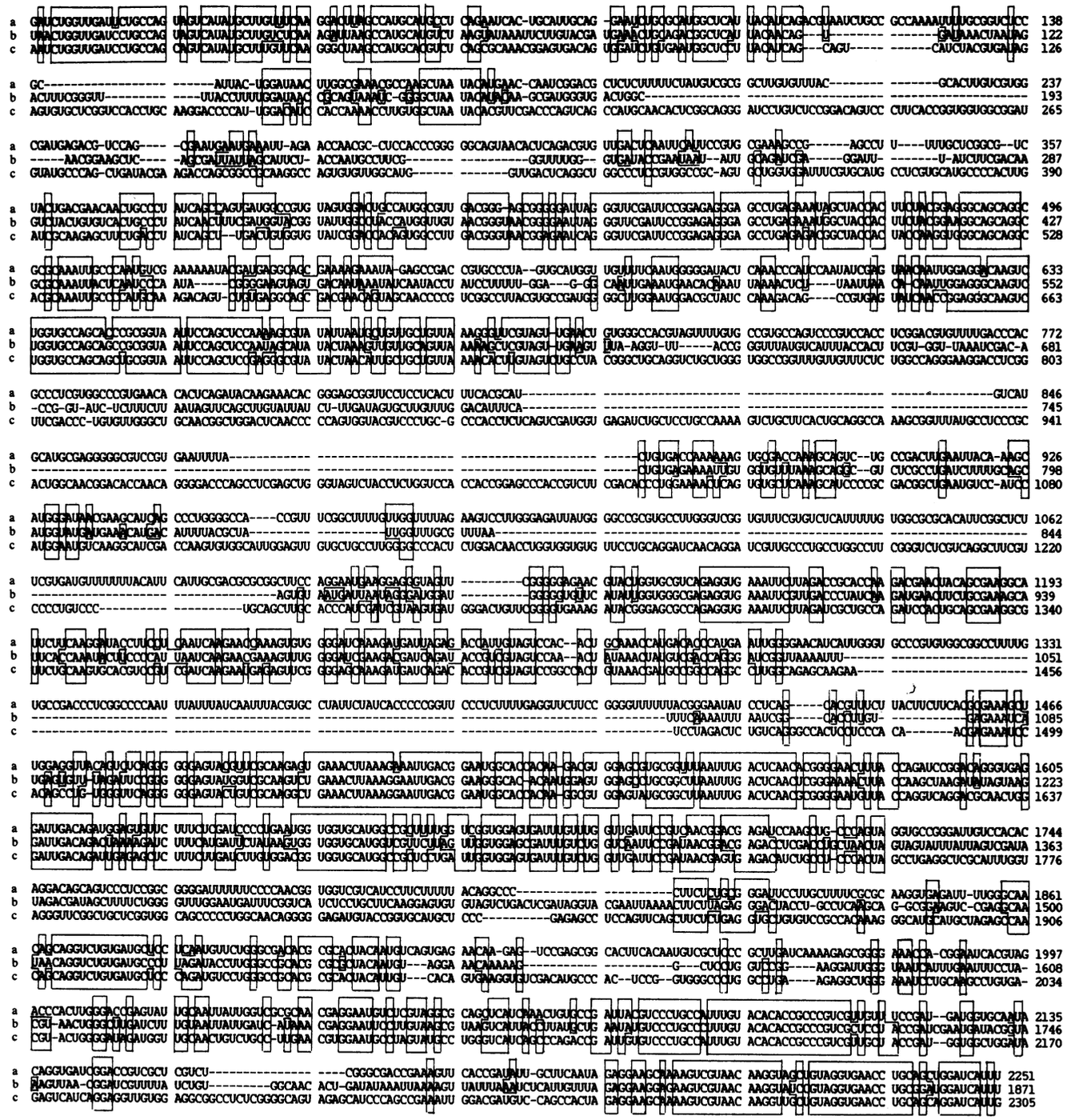

FIG. 1. Alignment of the small-subunit rRNA sequences from *T. brucei* (a) and *E. gracilis* (c) with that of *D. discoideum* (b). The sequences were initially aligned on the basis of obvious primary structure homology. The locations of phylogenetically conserved secondary structures were then juxtaposed to refine the alignments where length variation occurred. Boxed regions indicate nucleotide positions that are absolutely conserved in all known eukaryotic small-subunit rRNA genes. A number system for each sequence is provided in the figure.

are identified by their structural similarity to the highly conserved terminal regions found in other eukaryotes. The apparent lengths of the *T. brucei* and *E. gracilis* sequences are 2251 and 2305 nucleotides, respectively, which compares to representative eukaryotic small-subunit rRNA chain lengths of 1753 nucleotides for *T. thermophila* (17) and 1874 nucleotides for rat (26). The extra nucleotides are accounted for by three major insertions, which correspond to the *D. discoideum* positions 745, 844, and 1051. These regions display considerable length variation in all eukaryotic small-subunit rRNA sequences. The insertions may define introns, but electrophoretic and velocity sedimentation analyses have shown the *T. brucei* and *E. gracilis* small-subunit rRNAs to be larger than their evolutionary homologues in other eukaryotes (19, 33).

Table 1 shows the similarity values and the structural distances (number of substitutions per site) for comparisons of the small-subunit rRNA coding regions from *T. brucei* and *E. gracilis* with those of other eukaryotes as well as *Halobacterium volcanii* (34), *Sulfolobus solfataricus* (G. J. Olsen, personal communication), *E. coli* (36), and *Anacystis nidulans* (35). The analysis is restricted to a total of 1130 positions, which can be unambiguously aligned on the basis of obvious primary and secondary structure homology in all of the considered sequences. The *E. gracilis* and *T. brucei* sequences consistently display the lowest similarity to other eukaryotic small-subunit rRNAs. Furthermore, on the basis of rRNA similarity these two flagellated protists are not specifically affiliated with one another. The low degree of homology between the *T. brucei* and the *E. gracilis* sequences with their evolutionary homologues in other eukaryotes is reflected by base substitutions at positions that are invariant in the other eukaryotes. When *T. brucei* and *E. gracilis* are excluded from a consensus sequence analysis, the nucleotide usage is absolutely conserved at 856 positions. Of the 856 conserved positions, only 715 in the *E. gracilis* and 733 in the *T. brucei* sequences remain unchanged. The locations of the conserved positions are indicated in Fig. 2.

A modification of the distance matrix methods of Fitch and Margoliash (37) was used to convert the structural distance data into the phylogenetic tree shown in Fig. 2 (18). This phylogeny reveals that *T. brucei* and *E. gracilis* represent two deep but apparently separate branchings in the eukaryotic line of descent. (The *E. gracilis* and *T. brucei* branching points are too close to exclude the possibility that they diverged from one another very soon after their separation from the other eukaryotes.) The phylogenetic origins for the plants and animals correspond to a period of massive evolutionary radiation, which gave rise to the Fungi and a number of other protists including the ciliates and the acanthamoebae.

DISCUSSION

The rRNA similarity values in Table 1 and the phylogenetic tree in Fig. 2 illustrate a striking genetic diversity for the eukaryotes. Two deep independent branches represented by *T. brucei* and *E. gracilis* exceed the known depths of branching within the eubacteria or the archaebacteria. Although the homology data indicate that *E. gracilis* represents the earliest divergence from the mainstream of eukaryotic descent yet characterized by molecular phylogeny, our tree almost certainly underestimates the true diversity within the eukaryotes. Dinoflagellates, red algae, and *Pelomyxa* are likely to have diverged from the rest of the protists before *Euglena* did (1, 38). Rather than being a cohesive group equal in some phylogenetic way to the other major divisions, the protists are an assemblage of eukaryotic lineages; the diversity within the kingdom Protista dwarfs that of the Plantae, Fungi, and Animalia combined. A similar degree of protist diversity is seen in phylogenies based on 5S rRNA sequences (16), but the branching patterns are significantly different. For example, the ciliate divergence in our tree occurred much later than that of euglenoids and kinetoplasts. The low number of independently variable positions in 5S rRNAs contributes to a statistical uncertainty in the ordering of deep branches in phylogenetic trees.

The low homology of the *E. gracilis* and *T. brucei* sequences with other eukaryotes does not represent a rapid rate of evolutionary drift. If a sequence is subject to aberrant mutation rates, its homology with a distantly related sequence will differ from the homology of close relatives and the distantly related control sequence. As shown in Table 1, the homology between *E. coli* or *H. volcanii* and the flagel-

Table 1. Structural similarity and distance data between eukaryotic, eubacterial, and archaebacterial small-subunit rRNA gene sequences

Organism	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r
a		0.972	0.909	0.867	0.868	0.864	0.864	0.837	0.800	0.839	0.840	0.798	0.731	0.712	0.590	0.602	0.569	0.550
b	0.029		0.907	0.865	0.866	0.862	0.861	0.833	0.800	0.836	0.838	0.803	0.730	0.718	0.589	0.601	0.567	0.545
c	0.097	0.100		0.866	0.867	0.860	0.860	0.830	0.789	0.834	0.836	0.805	0.724	0.716	0.601	0.598	0.572	0.545
d	0.146	0.148	0.147		0.994	0.929	0.904	0.855	0.847	0.903	0.903	0.832	0.743	0.733	0.604	0.605	0.578	0.567
e	0.145	0.148	0.146	0.006		0.928	0.906	0.887	0.849	0.901	0.905	0.831	0.744	0.734	0.600	0.604	0.579	0.565
f	0.150	0.152	0.155	0.075	0.076		0.908	0.893	0.849	0.900	0.900	0.840	0.746	0.732	0.598	0.602	0.581	0.564
g	0.150	0.153	0.154	0.103	0.100	0.098		0.893	0.862	0.905	0.908	0.825	0.737	0.733	0.605	0.617	0.590	0.557
h	0.184	0.188	0.193	0.125	0.123	0.116	0.115		0.881	0.923	0.927	0.827	0.733	0.743	0.599	0.612	0.583	0.562
i	0.232	0.232	0.248	0.171	0.168	0.168	0.152	0.130		0.870	0.873	0.802	0.715	0.711	0.590	0.596	0.564	0.550
j	0.182	0.185	0.188	0.104	0.106	0.108	0.102	0.081	0.143		0.996	0.835	0.739	0.740	0.602	0.608	0.581	0.577
k	0.180	0.183	0.185	0.104	0.102	0.108	0.098	0.077	0.139	0.004		0.836	0.740	0.742	0.601	0.609	0.583	0.577
l	0.235	0.228	0.226	0.190	0.192	0.181	0.200	0.197	0.229	0.186	0.185		0.737	0.719	0.597	0.611	0.569	0.568
m	0.334	0.335	0.344	0.315	0.314	0.311	0.324	0.329	0.359	0.321	0.319	0.325		0.727	0.582	0.584	0.563	0.545
n	0.363	0.354	0.358	0.330	0.328	0.332	0.330	0.315	0.365	0.319	0.317	0.352	0.340		0.585	0.610	0.560	0.554
o	0.594	0.596	0.569	0.564	0.571	0.576	0.562	0.575	0.592	0.567	0.569	0.579	0.611	0.605		0.759	0.671	0.649
p	0.567	0.569	0.575	0.561	0.564	0.567	0.537	0.545	0.579	0.556	0.552	0.548	0.607	0.551	0.290		0.697	0.672
q	0.641	0.645	0.635	0.621	0.618	0.613	0.594	0.610	0.653	0.614	0.610	0.640	0.655	0.664	0.433	0.388		0.817
r	0.687	0.700	0.699	0.646	0.650	0.653	0.670	0.659	0.687	0.623	0.623	0.643	0.699	0.677	0.474	0.432	0.210	

The upper right half of the table gives structural similarity values (18) for regions that can be unambiguously aligned in all of the considered small-subunit rRNA sequences. A total of 1160 positions were considered. The structural distances (average number of base changes per sequence position) are shown in the lower left half of the table. Sequence data are from the following organisms: a, rat (26); b, *X. laevis* (28); c, *A. salina* (29); d, *Z. mays* (30); e, rice (31); f, *A. castellanii* (unpublished data); g, *S. cerevisiae* (32); h, *P. tetraurelia* (unpublished data); i, *T. thermophila* (17); j, *S. pustulata* (18); k, *O. nova* (18); l, *D. discoideum* (12); m, *T. brucei*; n, *E. gracilis*; o, *H. volcanii* (34); p, *S. solfataricus* (G. J. Olsen, personal communication); q, *A. nidulans* (35); r, *E. coli* (36).

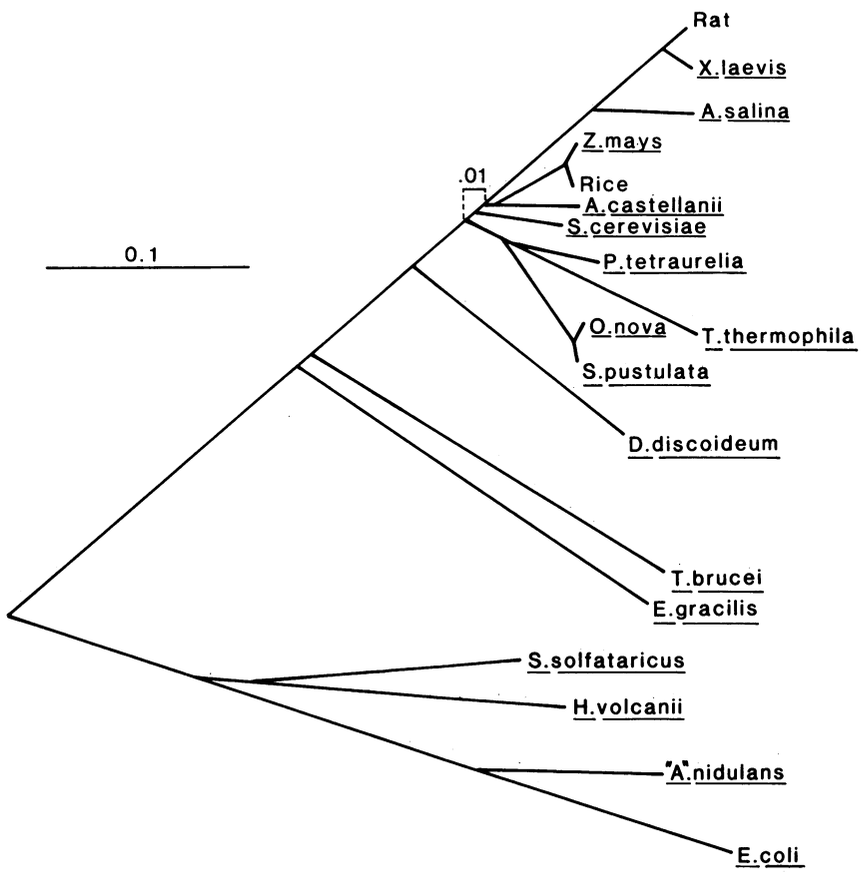

FIG. 2. Multikingdom phylogeny inferred from small-subunit rRNA sequence similarities. A phylogenetic tree was inferred by using the structural distance data in Table 1. The analysis is limited to ≈1130 positions. The tree was inferred by using the distance matrix methods of Fitch and Margoliash (37) as described (18). The evolutionary distance between nodes of the tree is represented in the horizontal component of their separation in the figure.

lated protists is similar to the homology between the other eukaryotes and *E. coli* or *H. volcanii*.

In contrast to the diverse phylogenetic origins of the different protist groups, the plant, animal, and fungal lineages originated during a relatively short period of time (as measured by rates of nucleotide substitutions in rRNAs), which coincided with the divergence of certain other protist groups including ciliates and acanthamoebae. At this time it is not possible to identify which present-day protists are most closely related to members of the other three eukaryotic kingdoms. The bracket about the tree at the point of radiation represents 1.0 nucleotide change per 100 positions; with such small differences branching orders cannot be accurately determined until a larger number of organisms are included in the analysis. One can only speculate about the changes in the ancestral protist group or environment that pressured these organisms to quickly radiate into such highly distinctive and adaptively separate lineages. The radiation might reflect a mass extinction that was followed by the creation of a wide spectrum of new ecological niches. Alternatively, new methods of organizing or processing genetic information may have developed that conferred upon these ancestral organisms a new and expanded evolutionary potential.

Several other features of our phylogenetic tree deserve comment. On the basis of rRNA sequence similarities, it is unlikely that the metazoa arose from the ciliates (39); the two groups are separated by forms that gave rise to the Fungi, higher plants, and probably a variety of other organisms. The position of *Euglena* in the tree is also noteworthy. The evolutionary distance between the higher plants and euglenoids is enormous, yet, on the basis of phenotypic characteristics produced by the plastids, euglenoids have frequently been placed close to the green algae, which gave rise to the higher plants (1). The discrepancy between plastid similarity and host dissimilarity could be explained if plastid characteristics were highly conserved or if similar plastids had been acquired separately by the two groups long after their evolutionary divergence. Finally, the relationship between the *Euglena* and *Trypanosoma* is of interest because euglenoids and kinetoplastids are sometimes united into a single phylum or supraphyletic assemblage (40). The rRNA similarity values for *E. gracilis* and *T. brucei* suggest a very distant relationship between the two and a long separate evolutionary history.

The phylogenetic tree shown in Fig. 2 is "unrooted;" however, groups of organisms that are known (by other criteria) to be related can be regarded as "subtrees" (12). The root of each subtree can be determined by sequence comparisons to organisms that lie outside the group. By using either the eubacterial or the archaebacterial sequences as out-groups, the deepest divergence in the eukaryotic line of descent yet characterized by comparisons of small-subunit rRNA sequences is represented by *E. gracilis*. There is no objective method for placing the root of the entire tree. We have arbitrarily placed the root so that the distance of the common ancestor to the three major lines of descent is similar for the eubacterial, archaebacterial, and eukaryotic representatives in the tree.

The eukaryotes are commonly regarded as organisms that evolved from forms related to present-day prokaryotes through the acquisition of discrete nuclei and the other organelles characteristic of extant eukaryotes. The eukaryotes are seen as a younger group directly descended from possibly still extant prokaryotic groups (41, 42). Alternative-

ly, it is possible to regard the eukaryotes (excluding those parts of the cell having an endosymbiotic origin) as a major line of descent that separated from the line leading to the modern prokaryotes (eubacteria and archaebacteria) before the level of complexity usually associated with the prokaryotic cell was even attained (43). The structures associated with modern eukaryotic cells (nuclei, microtubules, Golgi apparatus, mitochondria, chloroplasts) could have been acquired gradually and long after the eukaryotic line had separated from the prokaryotes. Our data can be construed as supporting the second alternative. The eukaryotic lines have not arisen from within the eubacterial or archaebacterial assemblages.

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1. Margulis, L. & Schwartz, K. V. (1982) *Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth* (Freeman, San Francisco).
2. Zuckerkandl, E. & Pauling, L. (1965) *J. Theor. Biol.* **8,** 357–366.
3. McLaughlin, P. J. & Dayhoff, M. O. (1973) *J. Mol. Evol.* **2,** 99–116.
4. Fitch, W. M. (1976) *J. Mol. Evol.* **8,** 13–14.
5. Yasunobu, K. T. & Tanaka, M. (1974) *Syst. Zool.* **22,** 570–589.
6. Rao, K. K. & Hall, D. O. (1977) in *The Evolution of Metalloenzymes, Metalloproteins and Related Materials*, ed. Leigh, G. J. (Symposium Press, London), pp. 39–64.
7. Rao, K. K. & Kammack, R. (1981) in *Molecular and Cellular Aspects of Microbial Evolution*, eds. Carlile, M. J., Collins, J. F. & Moseley, B. E. B. (Cambridge Univ. Press, Cambridge), pp. 175–213.
8. Asada, K., Kanematsu, S., Okada, S. & Hayakawa, T. (1980) in *Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase*, eds. Bannister, J. V. & Hill, H. A. O. (Elsevier/North-Holland, Amsterdam), pp. 136–153.
9. Fridovich, I. (1976) in *Free Radicals in Biology*, ed. Pryor, W. A. (Academic, New York), pp. 239–277.
10. Lumsden, J. & Hall, D. O. (1975) *Nature (London)* **257,** 670–672.
11. Gray, M. W. & Doolittle, W. F. (1982) *Microbiol. Rev.* **46,** 1–42.
12. McCarroll, R., Olsen, G. J., Stahl, Y. D., Woese, C. R. & Sogin, M. L. (1983) *Biochemistry* **22,** 5858–5868.
13. Yang, D., Oyaizu, Y., Oyaizu, H., Olsen, G. J. & Woese, C. R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4443–4447.
14. Hori, H. & Osawa, S. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 381–385.
15. Olsen, G. J. & Sogin, M. L. (1982) *Biochemistry* **21,** 2335–2343.
16. Ohama, T., Kumazaki, T., Hori, H. & Osawa, S. (1984) *Nucleic Acids Res.* **12,** 5101–5108.
17. Spangler, E. A. & Blackburn, E. H. (1985) *J. Biol. Chem.* **260,** 6334–6340.
18. Elwood, H. J., Olsen, G. J. & Sogin, M. L. (1985) *Mol. Biol. Evol.* **2,** 399–410.
19. Hasan, G., Turner, J. J. & Cordingley, J. S. (1982) *Nucleic Acids Res.* **10,** 6747–6761.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
21. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
22. Curtis, S. E. & Rawson, J. R. Y. (1981) *Gene* **15,** 237–247.
23. Williams, B. G. & Blattner, F. R. (1979) *J. Virol.* **29,** 555–575.
24. Sanger, F. & Coulson, A. R. (1975) *J. Mol. Biol.* **94,** 441–448.
25. Matteucci, M. D. & Caruthers, M. H. (1981) *J. Am. Chem. Soc.* **103,** 3185–3191.
26. Chan, Y., Gutell, R., Noller, H. F. & Wool, I. G. (1984) *J. Biol. Chem.* **259,** 224–230.
27. Connaughton, J. F., Rairkar, A., Lockard, R. E. & Kumaar, A. (1984) *Nucleic Acids Res.* **12,** 4731–4745.
28. Salim, M. & Maden, B. E. H. (1981) *Nature (London)* **291,** 205–208.
29. Nelles, L., Fang, B., Volckaert, G., Vandenberghe, A. & De Wachter, R. (1984) *Nucleic Acids Res.* **12,** 8749–8768.
30. Messing, J., Carlson, J., Hagen, G., Rubenstein, I. & Oleson, A. (1984) *DNA* **3,** 31–40.
31. Takaiwa, F., Oono, K. & Sugiura, M. (1984) *Nucleic Acids Res.* **12,** 5441–5448.
32. Rubtsov, P. M., Musakhanov, M. M., Zakharyev, V. M., Krayev, A. S., Skryabin, K. G. & Bayev, A. A. (1980) *Nucleic Acids Res.* **8,** 5779–5794.
33. Rawson, J. R. & Stutz, E. (1968) *J. Mol. Biol.* **33,** 309–314.
34. Gupta, R., Lanter, J. M. & Woese, C. R. (1983) *Science* **221,** 656–659.
35. Tomioka, N. & Sugiura, M. (1983) *Mol. Gen. Genet.* **191,** 46–50.
36. Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4801–4805.
37. Fitch, W. M. & Margoliash, E. (1967) *Science* **155,** 279–284.
38. Taylor, F. J. R. (1976) *J. Protozool.* **23,** 28–40.
39. Hanson, E. D. (1977) *The Origin and Early Evolution of Animals* (Wesleyan Univ. Press, Middletown, CT).
40. Corliss, J. O. (1984) *BioSystems* **17,** 87–126.
41. Cavalier-Smith, T. (1975) *Nature (London)* **256,** 463–468.
42. Margulis, L. (1981) *Symbiosis in Cell Evolution: Life and Its Environment on the Early Earth* (Freeman, San Francisco).
43. Fox, G. E., Stackebrandt, E., Hespell, R. B., Gibson, J., Maniloff, J., Dyer, T. A., Wolfe, R. S., Balch, W. E., Tanner, R. S., Magrum, L. J., Zablen, L. B., Blakemore, R., Gupta, R., Bonen, L., Lewis, B. J., Stahl, D. A., Chen, K. N., Luehrsen, K. R. & Woese, C. R. (1980) *Science* **209,** 457–463.